Linking the Life History and Genetics of Splittail with Estuarine Processes

Frederick V Feyrer

Public Comments

No public comments were received for this proposal.

Collaboration Panel Review

Proposal Title

#0071: Linking the Life History and Genetics of Splittail with Estuarine Processes

Final Panel Rating
adequate

Collaboration Panel (Primary) Review

Collaboration:

Will the results of the collaborative effort be greater than the sum of its parts? Is it clear why the subprojects are part of a larger collaborative proposal rather than several independent smaller ones?

adequate
Not clear that this effort provides for a sythesis of the subprojects.

Interdependence And Integration:

Does the proposal have an example that clearly articulates the conceptual model of each subproject and how they link together as a whole? Are the boundaries of the study plans focused and cohesive, yet well delineated? Is there a plan for potential differences in the stages of subproject completion times? Are there clear plans for analyses and interpretations which seek to identify and quantify relationships among the data collected in various subprojects rather than separate analyses for each subproject?

adequate
Yes, conceptual models are present for both genetics and otolith work. I did not find a discussion of a plan for differences in completion times nor a plan for analyses,

Project Management:

Is it clear who will be performing management tasks and administration of the project? Are there resources set aside for project management and time given for investigators to

collaborate? Is there a process for making decisions during the course of the project? Are there acknowledgments of potential barriers to collaboration and explanations of how team members will overcome barriers particular to their institutions?

adequate
Yes, it is clear who is performing project management and administration, though it does not appear that resources have been set aside for project management nor has time been given for collaboration. The is not a description of a decision process nor a process to overcome barriers.

Team Composition:

Does the lead principal investigator have successful management history and experience leading collaborative teams? Is it clear that all key personnel are committed to making significant contributions to the project? Do team members have complementary skills?

adequate
It is not clear that the PI has a successful history of managing a collaborative team of this nature. All key personnel seem committed to contributing to this project and have complementary skills.

Communication Of Results:

Is there a clear plan for comprehensive and cohesive reporting of project progress to the CALFED community?

adequate
Reporting will consist of quarterly progress reports, oral and poster presentations at the CALFED Science Conference, at least one manuscript for each hypothesis (3), and a variety of other presentations at meetings, workshops(not identified), and gill net data to be posted on BDAT.

Additional Comments:

Collaboration Panel (Discussion) Review

Primary review: Subprojects were identified, but synthesis is not clear. Conceptual models presented for genetics and otolith work, but no adequate disciptions of integration. No plans for analysis or integration. No budget resources, no time given for collaboration, problem solving, etc. Not clear if the PI was successful in past. Key personnel had complementary skillls and seemed committed. Communication products: one manuscript for each hypothesis (3), gill net data posted to the web. No funds for communication products. Tasks could have been subcontracted. Lack of synthsis discussions. Project of this size would need more discussion of analysis was needed.

Individual rating: Adequate

Secondary review: Positives: project management (work breakdown, task integration w/ timeline). Final manuscript deadline identified. Combining otolith chemistry and genetics fit together inherently. Straightforward integration by design. Split between Above average and Adequate. Evidence that PI has managed large studies. Weakness: not clear who the participating investigators are.

Individual rating: Above average

Combined rating: Adequate

Technical Synthesis Panel Review

Proposal Title

#0071: Linking the Life History and Genetics of Splittail with Estuarine Processes

Final Panel Rating
adequate

Technical Synthesis Panel (Primary) Review

TSP Primary Reviewer's Evaluation Summary And Rating:

Above average-Adequate Proposal addresses new discovery of increased population structure and possible estuarine dependency in splittail. Estuaries are much more extensive than fw rearing habitats. Therefore, understanding their role in production of splittail is critical in the recovery of this species of concern. Through directed sampling in the San Francisco Estuary, the PI s look to investigate the relative estuarine dependency – determined by otolith microchemistry – of three different populations (Petaluma R, Napa R, and Rivers of Central Valley) -identified through genomic analysis. The contribution of differing populations to common estuarine nursery areas can thus be determined across individuals and within individuals, lifetime patterns of estuarine dependency evaluated. Growth rates and fecundity will be related to patterns of habitat use. Otolith chemistry applications will be supported by an intensive laboratory experiment, where fish will be exposed for 60 days to different temperatures and salinities; and in 1-yr exposure studies that emulate natural conditions of salinity that a fish sees during its first year of life. The PI s also propose an ancillary analysis of fish community attributes from gill net sampling directed to collect adult splittail, a community of larger nekton that is undersampled in ongoing monitoring and research programs.

Additional Comments:

Integrated directed sampling, genetic analyses, and otolith microchemistry is strongest element of the proposal, which could provide insight on estuarine dependency and life history of this species of concern. Hypotheses are well stated and feasibly tested. Proposal itself lacked critical details on how individuals would be classified by population or contingent, how statistics would be applied to otolith chemistry studies, gill net deployments and analysis of community analysis. Given level of required integration and expense of project, more attention to explicating the approach seems required. The budget seems excessive balanced against likely research outcomes and products. Otolith chemistry section did not reflect the current literature or understanding of how strontium is affected by salinity gradient. No explication of statistical approaches was given throughout. Gill nets are notoriously size selective and in longer deployments (30 min) sets can cause undue mortality, particularly at warmer temperatures. No information was provided on what mesh sizes would be fished, nor how samples would be corrected for mesh size selectivities. The community analysis seemed largely unrelated to the aims of the proposal.

Above average-Adequate Proposal addresses new discovery of increased population structure and possible estuarine dependency in splittail. Estuaries are much more extensive than fw rearing habitats. Therefore, understanding their role in production of splittail is critical in the recovery of this species of concern. Through directed sampling in the San Francisco Estuary, the PI s look to investigate the relative estuarine dependency – determined by otolith microchemistry – of three different populations (Petaluma R, Napa R, and Rivers of Central Valley) –identified through genomic analysis. The contribution of differing populations to common estuarine nursery areas can thus be determined across individuals and within individuals, lifetime patterns of estuarine dependency evaluated. Growth rates and fecundity will be related to patterns of habitat use. Otolith chemistry applications will be supported by an intensive laboratory experiment, where fish

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Technical Synthesis Panel (Discussion) Review

TSP Observations, Findings And Recommendations:

The proposal received high marks from the external reviewers; however, the panel felt that the external reviewers were more impressed with the techniques than with the analyses in the proposal itself. The panel thought that the integrated directed sampling, genetic analyses, and otolith microchemistry were the strongest elements of the proposal, and could provide insight on estuarine dependency and life history of this species of concern. In general, the panel felt that the study would produce valuable life-history information but it was not clear how this information would be useful to fish and ecosystem management activities. The lack of background on the statistical analyses that will be used was considered to be a flaw in the study. This and other flaws in the study were considered to be reperable, but the authors did not make use of the space provided to address issues that the panel felt could and should have been addressed. The proposal would have benefited from incorporation of information from the "in press" study on splittail genetics and increased explanation on how individual fish would be classified into populations and different classes of past nursery habitat use. The budget was considered to be high for the anticipated products.

Technical Review #1

proposal title: Linking the Life History and Genetics of Splittail with Estuarine Processes

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	The primary goals and objectives are well stated and internally consistent, and include well-defined null hypotheses for statistical testing. A second objective, involving analysis of the fish community (apparently a by-product of the main activities, as they will be capturing other species besides the target) is less well defined. The project builds on recent genetic studies of splittail that suggest important avenues for the kind of life history/ecological study proposed here. The information gathered in this study should prove useful for general knowledge about the San Francisco estuary.
Rating	very good

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	The conceptual model is well described and is a reformulation of a previous life history model that needs to be updated due to recent discoveries about the genetic structure of splittail populations. Thus the proposed study seems to be a natural next step based

	upon existing knowledge. The proposal is described as a full-scale implementation but may need further sampling to understand annual variation. At this point the study is based on two years of sampling, but it is not clear that this is long enough to encompass relevant annual variation, particularly since the study organism is long-lived, according to the proposal. Full-scale implementation of their otolith chemistry work seems warranted, as they have already completed substantial exploratory/pilot research.
Rating	good

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	The approach seems well designed. It is a nice integration of field-based population and life history sampling, genetic identification of population structure, and ecological information (salinity history) based on otolith chemistry technology. While some of the techniques proposed may seem 'flashy' they are well suited to the questions the study seeks to answer. Based on the limited information in the proposal about genetic identification of populations using assignment tests, it is difficult to determine if that approach is feasible (i.e., they do not provide information about how variable their microsatellite loci are or how this variation is distributed across the three sub-populations they seek to assign individuals to). However, they cite previous research that seems to suggest that the three sub-populations are well defined genetically. They have already worked out many issues with the otolith chemistry work and have determined that otolith

chemistry correlates with some environmental variables. Also, for the otolith chemistry they propose to conduct a validation experiment with fishes held in controlled conditions – an important component which will also provide the groundwork for future studies.

The secondary objective, a community-wide study of the distribution and environmental correlates of fishes living in the estuary is less well described. The objectives and statistical techniques are vague.

I have two main concerns with the approach. First, I would like to see what steps they have taken to minimize the amount of non-target individuals killed by gill nets. Are there T species that may be killed? Are the mesh sizes they use designed to avoid accidental take of T species? The field effort is intensive (~1500 sets over two years), which is necessary for their study, but also seems like it may be killing a lot of fish. There is no information in the proposal about this, so it makes it hard to evaluate the benefit of the study compared to the cost in terms of by-catch. At least they propose to conduct a community-based distribution and ecology study with the by-catch data. Second, with the life history data they need to collect age-at-maturity and longevity data in addition to growth and fecundity. The importance of growth and fecundity depends on the schedule of reproduction, so to understand the relevance of inter-population differences in growth and fecundity for population productivity, these variables need to be analyzed in a life table framework (see Stearns 1992 The evolution of life histories).

In addition to the salinity history reconstructed from individual fish captured at known locations, they do not mention collecting environmental data at their gill net sets. This seems like an obvious thing to do if they are trying to connect distribution and life

	history data with salinity, and would be an important component of their community-wide study. (It sounds like they plan to collect environmental data - they have budgeted for a YSI - but it is not explicitly stated or described.) The study would definitely provide important data for splittail and reasonably good data about other fishes in the estuary, and thus would be useful to managers.
Rating	very good

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	See previous comments. The scale seems consistent with the overall objectives and the authors seem well qualified to conduct the research. As stated above, I am concerned that two years is too short to capture annual variation - but they can evaluate that at the end of the study (i.e. do they get conflicting results in the two different years?) and perhaps can continue the study if it seems warranted.
Rating	good

Monitoring

If applicable, is monitoring appropriately designed (pre–post comparisons; treatment–control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	Not applicable.
Rating	not applicable

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	The products include peer-reviewed publications, which are valuable to managers as well as the scientific community. They also plan to disseminate information and ideas at conferences, workshops, and public forums that include stakeholders. They have plans for making the data available on BDAT, which will also be useful to biologists and managers. However I would also like to see mention of depositing collected fishes in a research collection.
Rating	good

Additional Comments

Comments

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	The proposal is a well-designed collaboration between investigators with different (and complementary for the proposal) expertise. Each PI has an impressive list of publications that demonstrate their experience and ability to perform the proposed work. The infrastructure available to the PIs should be sufficient.
Rating	very good

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	The budget seems reasonable for the most part. However, they have budgeted a total of $15,000 between the three PIs for travel to national conferences. Is this really necessary? The value of the study seems fairly local (information about splittail and the San Francisco estuary) so I am not sure that CALFED needs to send all three to national conferences.
Rating	good

Overall

Provide a brief explanation of your summary rating.

Comments	Overall this is a strong proposal. The objectives are well formulated and should provide information pertinent to the CALFED objectives. The authors are knowledgeable and well qualified to conduct the proposed research. I am mainly concerned about the high cost in terms of by-catch in gill nets and the lack of a life table analysis of the growth and fecundity data. Also, the community-wide analysis of the gill net data is vague and seems like an afterthought, but as no real money is budgeted beyond what is being sought for the primary objective, they might as well conduct it.
Rating	very good

Technical Review #2

proposal title: Linking the Life History and Genetics of Splittail with Estuarine Processes

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	The proposal aims to evaluate linkages between genetics, habitat use (salinity exposure) and life history for splittail, a species of concern. A second objective is evaluate habitat associations for the fish community in the brackish region of the estuary. The authors will sample fish from three general areas that host 3 genetic strains of splittail. Specifically, the work will evaluate the following hypotheses: 1) Do the three distinct populations exhibit overlapping or segregated estuarine distributions? 2) Do the three populations experience different salinity regimes as measure from otolith microchemistry? 3)If splittail experience different salinity regimes, do these differences translate to associated effects on life history traits? They also aim to evaluate fish commmunity structure as it relates to relates environmental variables.

The linkages between genetics, habitat use and life history provides an excellent framework for this research. Ultimately these data will allow the authors to evaluate if the populations have overlapping or discrete distributions. They state that this has important management implications, but do not explicitly state how this information might |

	`influence specific management actions.`
Rating	`good`

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	`The research goals are reasonable, but the exact`
	`management implications are not explicitly stated. For`
	`instance, if the three populations have discrete`
	`distributions, how does this influence current`
	`management approaches?`

Rating	`very good`

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	`The multi-faceted nature of the work is to applauded,`
	`but the linkages between the different components are`
	`not always explicitly stated. For instance, fish will`
	`be fin clipped and subsequently and assigned back to`
	`its original population based on microsatellite`
	`markers. The genetic distinctiveness of the San`
	`Joaquin/Sacramento population from the other two`
	`populations is considerable, but the difference`
	`between Napa and Petaluma is much less so. This would`
	`suggest that there is an increased probability of`
	`mis-assignments. For instance, it would be useful to`
	`know if the populations have high frequency private`
	`alleles that would increase the probability of`
	`correctly assigning individuals to the correct`
	`population. Ultimately these data will allow the`

	authors to evaluate if the populations have overlapping or discrete distributions. A subset of fish will be retained for life history and microchemistry otolith analyses. The initial sample size seems adequate (n=50 / site), and the authors plan to use the data from this sample to determine the sample size for subsampling in the second year. The controlled experiments to evaluate the relationship of salinity and temperature on otolith microchemisty are an excellent idea and should provide a good baseline for interpreting the otolith data from wild fish. The experiment calls for a good range of temperatures and salinities, but the replication could be slightly higher. Estimates of fecundity based on ova counts could be supplemented with an estimate of the gonadosomatic index as well as fat extractions. This would provide a more comprehensive assessment of fish condition. Probably the weakest part of the proposal concerns the evaluation of fish community structure as it relates to habitat characteristics. For instance, it is not clear what environmental variables will be evaluated. Although this information could be useful, this part of the project sounds like a supplemental benefit of doing the work as opposed to a central objective.
Rating	very good

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	The work is feasible for the given time line. The lab experiments should be especially useful for helping interpret the field samples (otolith microchemistry). It would be nice to have more details on the genetic assignment methodology.
Rating	very good

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Monitoring

If applicable, is monitoring appropriately designed (pre–post comparisons; treatment–control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	The project will provide a baseline for understanding the population structure of splittail in the study area. Of particular interest is whether different populations have discrete or overlapping distributions. Also of interest is whether exposure to high salinity influences life history characteristics.
Rating	very good

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	The PIs plan to publish the work in peer reviewed journals. This is an excellent idea as it will result in products of superior quality.
Rating	excellent

Additional Comments

Comments	

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	Freyer, Moyle and May have extensive research and publishing records and are well suited to conduct this research.
Rating	

	excellent

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	The budget seems appropriate for the proposed work.
Rating	excellent

Overall

Provide a brief explanation of your summary rating.

Comments	The general approach is to be applauded. The combination of experimental work and field work should provide a good measure of how fish are using the existing habitat and how this history influences life history characteristics. However, additional details on methodology would be useful. Also, it would be helpful to have an idea of the specific management implications of the research.
Rating	very good

Technical Review #3

proposal title: Linking the Life History and Genetics of Splittail with Estuarine Processes

Review Form

Goals

Are the goals, objectives and hypotheses clearly stated and internally consistent? Is the idea timely and important?

Comments	The authors propose to examine variability in estuarine distributions and salinity histories in 3 genetically distinct populations of splittail within the San Francisco Bay - Delta region. They will also use data from an intensive sampling plan to to examine community dynamics of fishes in the study area. In general I found that the specific objectives and hypotheses to be clear and consistent. The finding of genetically distinct populations of splittail in the Delta is exciting and clearly worthy of further study. I found the inclusion of both genetics and otolith chemistry to be particularly useful, as I can see that the two approaches can provide complementary information that will lead to powerful tests of the hypotheses proposed here. Certainly the ideas framed here are timely, and given that splittail is a target species for CBDA.
Rating	excellent

Justification

Is the study justified relative to existing knowledge? Is a conceptual model clearly stated in the proposal and does it explain the underlying basis for the proposed work? Is the selection of research, pilot or demonstration project, or a full–scale implementation project justified?

Comments	I found that the authors made a compelling case for the work that they are proposing here. There is

	clearly a significant knowledge gap, identified by the CBDA, in terms of the importance of brackish water habitats to fish populations in the Delta region. Splittail would seem to be a logical model species for this work.
Rating	very good

Approach

Is the approach well designed and appropriate for meeting the objectives of the project? Is the approach feasible? Are results likely to add to the base of knowledge? Is the project likely to generate novel information, methodology, or approaches? Will the information ultimately be useful to decision makers?

Comments	I thought that the overall approach outlined here was sound, at least as far as you could tell from the proposal text. However, it should be noted that much of the rationale for the work rests on the finding of genetic differences among splittail populations in the Delta - work that has yet to be published in a peer-reviewed journal. And unfortunately we are given very little information concerning the results of the genetic analyses in the proposal. I would have liked to know the sample sizes used to generate Fig. 1. Also, some estimate of the degree of genetic differentiation among the populations would have been useful. We were not given the type of dissimilarity matrix that was used to generate the UPGMA cluster dendrogram. This is important because the authors propose two different genetic analyses here. The first involves determining the estuarine distributions of splittail in space and time. This will presumably be accomplished by mixed-stock analysis using an maximum likelihood approach that will provide the proportion of each of the three populations in a sample of splittail. This should be

relatively straightforward even with modest genetic differentiation among the populations. However, the second and third objectives will require that the population affinity of individual splittails be determined using the microsatellite markers. This is a MUCH more challenging task, requiring much larger genetic differences among populations. I am not sure how a reviewer was supposed to assess this component of the work based on 1 paragraph (7 lines!) in the laboratory activities section. Finally, it was notable that the authors gave no indication that they were planning on verifying that the genetic technique was actually working - i.e. they don't outline an independent method for determining population affinities of individuals that would verify the accuracy of the genetic data. Given that so much depends on this component, this seems a quite serious oversight.

The otolith chemistry section was described in much more detail. I thought that the authors outlined a solid experimental approach to investigating the effect of salinity on Sr/Ca ratios in otoliths. However, they should keep in mind that [Sr/Ca]water will likely have the most effect on [Sr/Ca]otolith, and not salinity per se. This is important because the Sr/Ca mixing curve between seawater and freshwater is non-linear, and generally almost all of the changes in Sr/Ca with salinity occur below 10ppt. Strontium isotopes will show a similar pattern, although the increased precision of the technique (and the invariant nature of [87/86Sr]water and [87/86Sr]otolith relationship) may make this a better bet. In any case the authors need to be aware that salinity estimates are likely to be coarse

	and perhaps even binary (< or < 10ppt) with both techniques. Finally, the S in ICP-MS is spectrometry, not spectroscopy. Finally, I didn't couldn't find any mention of sample sizes to be examined, except for page 7 para 4 where the authors suggest that "Our initial target is fifty fish per population per year". This apparently refers to objectives 2 and 3, but what about objective 1? This will presumably require genetic analyses of many samples from different locations in the estuary, and sample size requirements here will swamp the 50 fish per population per year that are listed in the project.
Rating	good

Feasibility

Is the approach fully documented and technically feasible? What is the likelihood of success? Is the scale of the project consistent with the objectives and within the grasp of authors?

Comments	See comments above. The feasibility of the project rests on the ability of the microsatellite markers to be able to identify the population affinities of individual fish. However, the reviewers were given little way of assessing if this is realistic or not. And it doesn't appear that the authors have considered ways of verifying if the genetic techique is performing adequately. The otolith chemistry section was more compelling, with laboratory experiments to determine the effect of salinity and temperature on otolith chemistry, and then interpreting salinity histories of wild-caught fish based on the laboratory data.
Rating	good

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Monitoring

If applicable, is monitoring appropriately designed (pre–post comparisons; treatment–control comparisons)? Are there plans to interpret monitoring data or otherwise develop information?

Comments	Monitoring is not a specific component of the project here, but the authors seem to have given the experimental design of the collections planned here considerable thought. This should, in turn, allow them to both complete exploratory multivariate analyses of community structure, and provide samples for future projects.
Rating	not applicable

Products

Are products of value likely from the project? Are contributions to larger data management systems relevant and considered? Are interpretive (or interpretable) outcomes likely from the project?

Comments	If the research outlined here is successful, there will products of significant value resulting. Certainly the initial results of population genetic structure in splittail populations really does demand that further research be undertaken on this species in the Delta. Correlating estuarine habitat use (based on otolith chemsitry) with population affinity (from genetics) promising some exciting results. There intensive field effort will also produce contributions that are likely to be relevant to the larger efforts of the CBDA.
Rating	very good

Additional Comments

Comments	

Capabilities

What is the track record of authors in terms of past performance? Is the project team qualified to efficiently and effectively implement the proposed project? Do they have available the infrastructure and other aspects of support necessary to accomplish the project?

Comments	All three principal investigators have excellent records of past performance in their respecitve fields. Feyrer's position within the California Department of Water Resources should ensure that there are sufficient resources for the intensive field component of the study. May is an internationally recognized expert in the application of genomics to questions in aquatic ecology. Moyle is an excellent fish ecology with considerable experience in the study system. The PIs are supported by promising scientists providing expertise in population genetics and otolith chemistry. Instrument infrastructure at UCD should ensure that the authors have access to the instrumentation that they need to complete the project.
Rating	very good

Budget

Is the budget reasonable and adequate for the work proposed?

Comments	The authors are requesting almost $1m over 2 years for the research outlined here. If the figures are correct that they will analyse 300 fish in the 2 years, then the cost of this research is over $3,000/fish. Enough said. Much of the funds will go to the collection of samples, which is expensive and needs to be done. Indeed, it is difficult to suggest where these funds need to be cut, because most are for salaries. The granting agency will obviously need to decide funding levels if the proposal is successful, but it would seem that it would be essential to integrate this proposal with successful projects to ensure

	efficiency.
Rating	good

Overall

Provide a brief explanation of your summary rating.

Comments	Overall, I thought that the authors propose exciting research that will answer significant questions with regards the ecology of splittail and will likely be of considerable benefit to decision makers. From my comments it is obvious that I was a little frustrated at the lack of information regarding the genetic analyses. And at least taken at face value the proposal seems very expensive on a per-analysis basis. My immediate thought was is there some way that both genetic and otolith chemistry could be used to independently determine population affinity? For instance, if Sr isotopes in water are distinct among spawning areas of the three populations, could analysis of otolith cores be used to determine natal location? Perhaps the accuracy of the genetic analyses can be cross-validated somehow with fish of known population affinity (adults collected on each of the spawning locations? Early-stage larvae collected before dispersing from natal locations?). To conclude, I am very supportive of the project. Obviously some things will need to be considered before initiation of the work, but clearly this is a very competent group of researchers. The application of both genetic and otolith chemistry approaches should be particularly powerful - it often doesn't make sense to use both techniques but in this situation I think that it will be very useful.
Rating	very good